



Reproductive hormones profiles in Malnad Gidda cows during estrous cycle

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ABSTRACT

Malnad Gidda cows are famous for regular calving under low input regime. However, Malnad Gidda cattle are poorly studied with regard to their reproductive efficiency. The present study was undertaken to establish the baseline values for reproductive hormones in Malnad Gidda cows. Malnad Gidda cows (6) aged between three to four years were selected. Upon confirming the regular estrous cycles, blood was collected on the day of estrus (day 0) and the day 3, 6, 9, 12, 15, 18, 20 of estrous cycle and subsequent estrus (day 0). Serum was separated by centrifugation at 3,000 rpm at 30°C for 20 min and stored at -20°C until analyzed. 17β-estradiol and progesterone concentration in serum was measured using commercial radioimmunoassay (RIA) kit (BRIT, Mumbai). The serum 17β-estradiol hormone concentration showed a significant decrease from day 0 to day 3. Later, the concentration increased significantly up to day 6 and then decreased by day 9. The concentration of estradiol showed significantly increasing trend from day 12 and onwards up to day 20. The concentration of progesterone increased significantly from day 3 to day 9. Thereafter, the concentration decreased significantly from day 12 to day 20. The present study revealed the normal functioning of the Hypothalamo-Pituitary-Ovarian relationship axis which is responsible for normal estrous cycles in Malnad Gidda cows. The peak progesterone level was observed from 9th to 12th day and on the same days, the estradiol level was at its lowest levels.

Keywords: Estradiol, Estrous, Hormones, Malnad Gidda, Progesterone

In India, the breeding policy for dairy cattle have focused mainly on increasing productivity and have ignored the traits such as disease resistance, draught animal power, adaptability to extreme environmental conditions etc., present in the native Indian breeds. Malnad Gidda is one such dwarf breed of Indian cattle with their home tract in Southern India. There are 27 different types of Malnad Gidda varieties or strains having distinct characters and are found to be distinctly different from other breeds in the State of Karnataka (Anonymous 2009). It is a local breed of small to medium sized cattle with short and sturdy legs, short horns, and mainly distributed in Shimoga, parts of Chickmagalur, Hassan, North Canara, South Canara and Belgaum districts of Karnataka. They are well known for their capacity of survival in the hilly terrain and adverse climatic conditions of Western Ghats, resistance to parasitic infestations, diseases and need less fodder. Malnad Gidda cows are famous for regular calving under low input regime (Ramesha *et al.* 2013) and have shown more reproductive potentiality than others indigenous and crossbred in tropical climate condition. But, due to crossbreeding, this potential variety is in endanger condition at present. Therefore, reproductive management might be a process for

multiplying this cattle variety. Also, the reproductive hormones profile during estrous cycle is of most important for proper breeding strategies (Naik *et al.* 2013). However, Malnad Gidda cattle are poorly studied with regard to their reproductive efficiency. The studies on reproductive hormones have not been made in the past, hence there is need to establish and characterize reproductive prolificacy in Malnad Gidda cattle for their conservation and propagation (Murugeppa 2020). Accordingly, to establish the baseline values for reproductive hormones in female Malnad Gidda cattle, the present study was undertaken.

MATERIALS AND METHODS

For estradiol and progesterone estimation, a total of 6 Malnad Gidda cows aged between 3–4 years were selected. The animals were screened for regularity of estrous cycle and any other disease conditions. Upon confirming the regular estrous cycles, blood was collected on the day of estrus (day 0) and the day 3, 6, 9, 12, 15, 18, 20 of estrous cycle and subsequent estrus (day 0). The blood samples were collected without EDTA and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 3,000 rpm at 30°C for 20 min and stored at -20°C until analyzed.

17β-estradiol concentration in serum was measured using commercial radioimmunoassay (RIA) kit (BRIT, Mumbai) following the procedure as described in the kit.

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The assay quality control samples containing high and low hormone concentration were included in the beginning and at the end of each assay. Intra-assay and inter-assay coefficient of variations were 12.1 and 11.2%, respectively. The estradiol assay specificity was 100% and sensitivity was 1 pg/mL. Progesterone concentration in serum was measured using commercial radioimmunoassay (RIA) kit (BRIT, Mumbai) following the procedure as described in the kit. The assay quality control samples containing high and low concentrations were included in the beginning and at the end of each assay. Intra-assay and inter-assay coefficient of variation were 6.5 and 7.2%, respectively. The assay specificity was cent per cent and its sensitivity was 0.1 ng/mL. The data were analyzed with the aid of computerized statistical software, GraphPad Prism Version 5.01 (2007) by applying two-way ANOVA at 0.05% level of significance with the application of Tukey's post test. Mean values and standard error of mean were calculated and all the values are expressed as Mean±SE (GraphPad Prism 2007).

RESULTS AND DISCUSSION

The serum 17- β estradiol and progesterone concentrations during estrous cycle, on the day of estrus (day 0) and on day 3, 6, 9, 12, 15, 18, 19, 20 of estrous cycle and subsequent estrus (day 0) are presented in Table 1.

The values of 17 β -estradiol hormone concentration obtained in the present study were in agreement with the values obtained by Shukla *et al.* (2000) in crossbred cows at different days of estrus cycle. However, the higher 17 β -estradiol concentrations on different days of estrus cycle than the present finding was recorded by Singh *et al.* (2006) in Sahiwal crossbred heifers, Tabatabaei *et al.* (2014) in Holstein cattle, Pemayun *et al.* (2016) in Bali cattle and Kerketta *et al.* (2019) in crossbred cattle. But, Purohit *et al.* (2000) in indigenous Rathi cows and Kim (2018) in dairy cows recorded lower levels than present findings. The variations in the values of 17 β -estradiol concentration reported by the earlier studies could be due to the variations

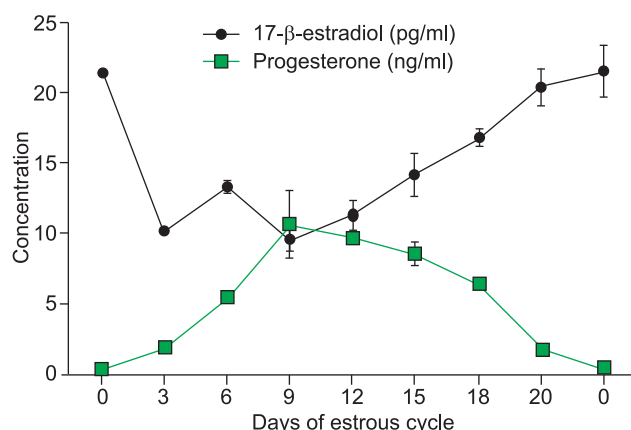


Fig. 1. Serum 17 β -estradiol and progesterone concentrations during estrous cycle.

Table 1. Serum 17 β -estradiol (pg/ml) and progesterone (ng/ml) concentrations during estrous cycle in Malnad Gidda cows

Day	17 β -estradiol (pg/ml)	Progesterone (ng/ml)
0	21.42±0.02 ^a	0.39±0.01 ^d
3	10.16±0.37 ^b	1.79±0.27 ^d
6	13.35±0.57 ^c	5.42±0.52 ^c
9	9.58±0.80 ^b	10.59±2.40 ^a
12	11.26±1.10 ^b	9.68±0.69 ^a
15	14.21±1.45 ^c	8.58±0.86 ^b
18	16.73±0.61 ^d	6.42±0.49 ^c
20	20.42±1.29 ^a	1.76±0.22 ^d
Day 0	21.55±1.82 ^a	0.36±0.01 ^d

Values with different superscripts in a column differ significantly ($p < 0.05$).

in the sampling frequency, seasons and weather at the time of sampling, and age and physiological stages of the animals as opined by Alvarez *et al.* (2000).

In the present study, 17 β -estradiol hormone concentration showed a significant ($p < 0.05$) decrease on day 3 compared to day 0 of estrus cycle. This decrease in 17 β -estradiol concentration was due to ovulation and subsequent development of luteal tissue under the influence of luteinizing hormone (Noakes *et al.* 2001, Hafez and Hafez 2013). Later, the 17 β -estradiol hormone concentration on day 6 compared to day 3 of estrus cycle showed a significant increase. This increase of 17 β -estradiol concentration could be due to the development of first wave of follicular growth as evidenced by Ireland and Roche (1983). However, the 17 β -estradiol concentration on day 9 was decreased significantly compared to day 6 of estrous cycle, which could be assigned to the atresia of dominant follicle developed during first follicular wave (Noakes *et al.* 2001, Hafez and Hafez 2013). Further, the 17 β -estradiol concentration after day 9 onwards showed an increasing trend up to subsequent estrus day 0 and reached the peak concentration (Hafez and Hafez 2013, Pemayun *et al.* 2016). This peak level was due to completely developed Graafian follicle which produces estradiol hormone at high concentrations (Alvarez *et al.* 2000, Hafez and Hafez 2013). Similarly, Tabatabaei *et al.* (2014) noticed higher plasma concentrations of 17 β -estradiol in proestrus and estrus than the other stages of the estrous cycle. Also, observation by Pemayun *et al.* (2016), Kerketta *et al.* (2019) and Hassan *et al.* (2021) found increased serum 17 β -estradiol concentration from the day 17 until the appearance of sign of estrus. The pre-estrus rise in hormone stimulates the surge of LH from the anterior pituitary which is necessary for follicular maturation, ovulation and CL formation (Noakes *et al.* 2001). Hence, it could be concluded that the release of 17 β -estradiol hormone is pulsatile in manner and the endocrine changes of 17 β -estradiol hormone reflected the growth of follicles in a wave like fashion with two waves in an estrous cycle.

The concentration of progesterone increased significantly from day 3 to day 9. Thereafter, the

concentration decreased significantly from day 12 to day 20. The values of progesterone hormone concentration on different days of estrous cycle obtained in the present study were in accordance with the findings of Singh *et al.* (2006) in Sahiwal; Kerketta *et al.* (2019) in crossbred cattle and Islam *et al.* (2020) in Red Chittagong cattle. However, report by Dhabale *et al.* (2000) in repeat breeding cows, Rabiee *et al.* (2002) in lactating cows at day 0 and Yildiz *et al.* (2005) in exotic cows and Kim (2018) in dairy cows at different days of estrous cycle were higher than the present study findings. Whereas the reports by Shukla *et al.* (2000) in crossbred cows, Tabatabaei *et al.* (2014) in Holstein cattle, Agarwal *et al.* (1989) in repeat breeding cows, Mondal and Prakash (2003) in Sahiwal cows, Patel *et al.* (2006) in HF cows and Madureira *et al.* (2021) in Holstein cows were lower than the present findings.

In the present study, no significant ($p > 0.05$) difference in the progesterone hormone concentration was observed on day 0 and day 3 of estrous cycle. But, the progesterone hormone concentration on day 6 was significantly ($p < 0.05$) higher than day 3 of estrous cycle. Later on day 9 of estrous cycle, the significantly ($p < 0.05$) highest progesterone concentration was observed and it could be attributed to the fully functional corpus luteum which produces progesterone hormone at high concentration (Noakes *et al.* 2001). Then after from day 12 onwards, the progesterone concentration showed significant ($p < 0.05$) decrease and finally reached the lowest concentration on subsequent estrus day 0 of next estrous cycle. The mean concentrations of progesterone increased positively and linearly from day 0 until day 12 of the oestrus cycle and started to decrease at day 15, indicating luteolysis (Hassan *et al.* 2021).

The concentration of progesterone in peripheral plasma reflects the luteal function in cows during the normal estrous cycle. Similarly, Tabatabaei *et al.* (2014), Kerketta *et al.* (2019) and Islam *et al.* (2020) noticed higher progesterone concentration in diestrus than in proestrus, estrus and during metestrus stage of estrous cycle. The post-estrous higher concentration of progesterone hormone up to day 18 could be due to luteinisation of follicular cells after ovulation and subsequent progesterone secretion from the corpus luteum. The subsequent decreased pattern from day 18 was due to the initiation of luteolysis (Hafez and Hafez 2013). The fall of progesterone hormone concentration to the lowest basal levels removes the anterior pituitary block and allows the sudden release of gonadotrophins (Noakes *et al.* 2001).

The present study revealed the normal functioning of the Hypothalamo-Pituitary-Ovarian relationship axis which is responsible for normal estrous cycles in Malnad Gidda cows. The peak progesterone level was observed from 9th to 12th day and on the same days, the estradiol level was at its lowest levels. From the established literature (Hopkins 2003), it is evident that the highest concentration of progesterone reaches just prior to initiation of CL regression and that peak coincides with the lowest concentration of estradiol which later on increases due to follicular growth as result of surplus of gonadotrophins levels. Similarly, in

the present study, the progesterone peak was observed from 9th to 12th days and on the same day, the 17 β -estradiol level was at its lowest level which indicated the early initiation of regression of CL in these animals with longer follicular phase because of early production of proglandins. The present study revealed the normal functioning of the Hypothalamo-Pituitary-Ovarian relationship axis which is responsible for normal estrous cycles in Malnad Gidda cows. The knowledge on the hormonal profile at various stages of estrous cycle is important for the selection and successful implementation of the estrus synchronization and other breeding programme.

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